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Tumor antigens in human cancer control

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ABSTRACT

The body of evidence that is supporting the role of T cells in human tumor control is substantial and it is now beyond doubt that T cells can be crucial in the clinical response to cancer immunotherapies such as adoptive T cell therapy and checkpoint blockade. This has been proven in particular for melanoma and non-small cell lung cancer. Strikingly, while clinical experience with these therapies is extensive, what these T cells detect on the tumors remains largely unknown. An extensive effort has been put into the characterization of tumor antigens and based on the recent successes of immunotherapies Cancer/Germline, mutated and viral antigens appear rather promising targets for tumor control. Furthermore, it is becoming evident that the most potent antigen in tumor control is highly dependent on the type of malignancy and may also vary even within malignancies.

1. Why T cells are relevant

It is now beyond doubt that the endogenous T cell based immune system can recognize cancer cells and in some situations control the disease. Pre-clinical data from numerous mouse models have demonstrated the importance of T cells in tumor control by depleting either the adaptive immune response or T cells alone resulting in abrogation of tumor rejection to various degrees. The importance of T cells in the human setting is supported by the correlation of tumor infiltrating T cells with good prognosis in a substantial number of different cancers [1–4]. In particular, it is well described that the location and activation status of T cells in colorectal cancer is of high prognostic value. Galon and colleagues have shown that quantifying the infiltration of antigen experienced $CD8^+$ T cells in the tumor invasive margin and the center of the tumor as a prognostic tool is equally strong (or potentially even better) as the currently used staging system [5,6]. Furthermore, work from Ribas and colleagues has shown that tumor infiltration and location of $CD8^+$ positive cells in human melanoma can function as a predictive biomarker for clinical outcome to anti-PD-1 therapy [7]. Nevertheless, the mere infiltration of T cells into cancer and the correlation with prognosis or clinical outcome are not providing direct evidence for T cells being an active contributor to the control of human cancer.

As a direct proof for the tumoricidal potential of patient autologous T cells, Rosenberg and colleagues have shown that infusion of autologous ex vivo expanded tumor infiltrating lymphocytes (TILs) can induce objective clinical responses in melanoma patients [8,9]. Similar response rates are achieved when infusing TIL products enriched for $CD8^+$ T cells providing direct evidence for the tumor killing capacity of these cells [10–12]. Within the last few years, the evidence for the activity of T cells in tumor control has extended beyond melanoma to a number of other human malignancies. This has in particular been demonstrated in clinical trials showing responses to anti-PD-1 therapy in a number of cancers including non-small cell lung cancer (NSCLC), bladder cancer, renal cell carcinoma and Hodgkin's lymphoma [13–16]. Furthermore, it was shown in a recent case report that TIL therapy in a patient with metastatic cholangiocarcinoma was able to mediate tumor regression [17].

The main point that can be inferred from these clinical trial data is that a proportion of tumor cells must express antigens that allow endogenous T cells to specifically recognize and kill them. Having established that T cells can play a pivotal role in human cancer control, the next step is to assess which antigens that can be recognized by T cells leading to tumor regression.

2. Which antigens are detected

T cells can recognize antigens that are presented on the surface of tumor cells in the context of HLA class I and II molecules and thereby mediate tumor cell destruction. These stretches of antigen bound to HLA molecules, epitopes, are a result of protein degradation in the cytosol. The fragmented antigens are transported into the ER lumen where they can be loaded onto an HLA molecule that will be translocated to the surface of the cell. Therefore, the epitopes bound to the HLA molecule forms a representation of cellular content. The first appreciation

Abbreviations: C/G, Cancer/Germline; CTLA-4, cytotoxic T lymphocyte antigen 4; HERV, human endogenous retrovirus; HLA, human leukocyte antigen; HPV, human papilloma virus; MHC, major histocompatibility complex; NSCLC, non-small cell lung cancer; PD-1, programmed death receptor 1; PD-L1, programmed death receptor ligand 1; SEREX, serological analysis of cDNA expression libraries; TAA, tumor associated antigen; TCR, T cell receptor; TILs, tumor infiltrating lymphocytes; TME, tumor microenvironment; TSA, tumor specific antigen.

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of such antigens recognized by autologous T cells came from van der Bruggen and colleagues in 1991 with the identification of MAGE-A1 [18]. Since this first discovery a huge number of T cell epitopes have been characterized from proteins with an aberrant expression in tumor cells (Fig. 1).

The characterized T cell epitopes were identified through either the use of patient-derived T cell populations or by the 'reverse immunology' approach. Using the approach of dissecting what tumor reactive T cell clones from cancer patients recognize directly provides evidence for the immunogenicity of the identified epitopes. The reverse immunology strategy does not provide a similar validation and it is therefor central to demonstrate that T cell epitopes identified with such a strategy can be recognized by T cells from cancer patients directly ex vivo, and very importantly that these T cells recognize the autologous tumor.

Tumor antigens can be divided into two main classes. The first being the tumor associated antigens (TAAs), which includes proteins that are shared between tumor and healthy tissue and to which tolerance is incomplete. These antigens can be further divided into categories based on their expression pattern in healthy tissues. One category is the over-expressed antigens [19,20]. These are proteins expressed by various healthy tissues and tumor cells and differ at the expression level of the proteins. A second category is the cell lineage-specific group of proteins including the melanocyte differentiation antigens that are expressed by the vast majority of melanomas [21,22]. A third category is the Cancer/Germline (C/G) antigens. These proteins are encoded by genes mainly expressed in germline cells and can be re-expressed in tumor due to dysregulation of demethylation in tumor cells [23].

The second class of antigens is formed by the tumor specific antigens (TSAs) also referred to as neo-antigens. These antigens include mutated antigens that arise as a consequence of tumor specific DNA damage as well as antigens resulting from the expression of oncogenic viral proteins.

This review will mainly focus on C/G and neo-antigens and discuss the promising evidence for their clinical relevance in the context of cancer immunotherapy.

3. Cancer/Germline antigens

Proteins are stratified to the group of C/G antigens based on their expression in both germ line cells and cancers. The C/G proteins are widely expressed during fetal development but become silenced for a substantial part by methylation of the genes in adult tissue, except in germ cells. These genes are often re-expressed in cancers likely caused by changes in genomic methylation [23]. The first C/G antigen described was MAGE1 (now known as MAGEA1, Fig. 1). This antigen was identified using patient-derived tumor-reactive T cell clones isolated from a melanoma patient with an unusual favorable clinical course [18]. In the following decades >200 antigens belonging to this family have been identified. This discovery was in particular fast-tracked with the development of the SEREX technology (serological analysis of cDNA expression libraries).

The expression of C/G antigens varies greatly between different malignancies. As an example, NY-ESO-1 is expressed in NSCLC, ovarian carcinoma, breast carcinoma and melanoma with varying frequencies [24]. Furthermore, within each tumor lesion the expression of a certain C/G antigen can be highly heterogeneous. Hence, the expression of C/G antigens appears to be somewhat tumor 'private' even though it is a shared antigen.

The reason for the heterogeneous expression pattern of C/G antigens has not yet been established but might reflect an ongoing Darwinian pressure together with redundant functionality. For some C/G antigens it has been demonstrated that the expression can be beneficial for tumor cells. As an example, proteins of the MAGE family have been found to modulate cell survival by suppressing the function of p53 [25] and linked to acquired drug resistance in relapsing multiple myeloma patients [26,27]. However, for the vast majority of C/G antigens there is no clear function known and thus their expression may be a stochastic event caused by tumor related changes in chromosomal stability. Regardless of their function that may or may not be linked to survival benefit, these antigens still form a useful group of targets.

The restricted expression profile of C/G antigens in healthy tissue and their aberrant expression in various tumors have made this group of antigens theoretically highly attractive targets for anti-cancer immune interventions. This is reflected by the high number of early clinical trials that have used members of this protein family as targets. The vast majority of these trials was based on vaccination strategies and did not result in notable toxicities nor did they result in sufficient clinical efficacy to be further developed. Despite the conceptually attractive characteristics of these antigens there are three crucial points to consider before developing therapies that will steer potent immune responses towards them.

First, more recent studies mapping the expression pattern of these proteins revealed that a good number are expressed at varying levels on healthy tissues accessible to the immune system. Examples are MAGE-A9 expressed on brain tissue and MAGE-A11 on lung tissue [28]. Based on these data a proportion of antigens that have previously been regarded as tumor-restricted targets might potentially cause severe toxicities when targeted with potent immune interventions. As a matter of fact, such toxicities have been encountered in clinical trials making use of T cell receptor (TCR) gene-engineered T cells for adoptive cell therapy. Severe safety issues were observed in a clinical trial in which melanoma patients were treated with a MAGE-A3 specific TCR [29]. This TCR cross-reacted with an epitope derived from MAGE-A12, which is expressed at low levels in the gray matter of the brain. This resulted in severe brain toxicity in 1/3 of the treated patients. More encouragingly, an NY-ESO-1 specific TCR was used in a clinical trial without toxicity issues in melanoma patients and synovial cell sarcoma patients achieving high objective response rates [30]. Taken together,

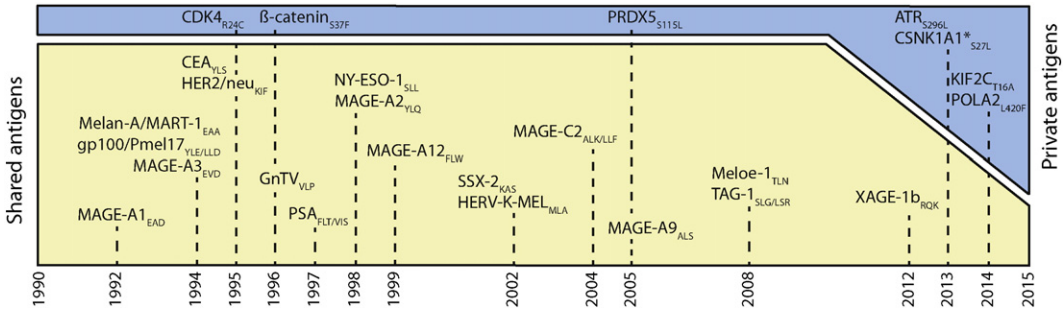

Fig. 1. Time frame of antigen discovery. Over the course of 25 years, a multitude of tumor antigens have been discovered using various technologies such as cDNA library cloning, SEREX, reverse-immunology and, more recently, whole tumor-exome sequencing. The colored parts contain a non-exhaustive list of tumor antigens for which T-cell responses have been detected, in yellow for shared antigens and in blue for private antigens, with a more dominant focus on the latter antigen class in recent years. Data was retrieved from the Cancer Immunity Peptide Database [74]. T cell responses detected against the neo-antigens ATR, CSNK1A1 and, KIF2C and POLA2 were retrieved from [40,41,42], respectively. Asterisk indicates that multiple neo-antigen specific T-cell responses were detected but only one here is shown.